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# Pilot Study and First Report on the Jelly Fish (Crambionella *Stuhlmanni*) Associated Bacteria and its Amylase Producing Properties

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**Abstract:** A total of 27 isolates of bacteria were isolated from the jelly fish Crambionella *stuhlmanni* was obtained from the Parangipettai fish landing centre, south east coast of India. The viable count of the bacteria in Crambionella *stuhlmanni* ranged from $10^3$-$10^6$ per g. The morphological and, biochemical investigations were done to characterize the isolates. Based on biochemical and morphological identification the isolates were found to be Vibrio, Staphylococcus, Lactobacillus, salmonella and Klebsiella species were predominant. All isolates were primarily screened for amylase production by starch plate method. Among 27 isolates, 2 isolates hydrolyzed the starch on starch agar and only one isolate was selected for further study.
**Key Words:** Biochemical, Morphological, Isolates, Amylase, Predominant.

## 1. Introduction:

Jellyfish are well-known for their unanticipated blooms of massive abundance. Some species of large jellyfish are widely used for Chinese cooking. Their medicinal value has also been known for a long time[1]. Jellyfish are found in every ocean, from the surface to the deep sea. Some hydrozoan jellyfish, or hydromedusae, are also found in fresh water; freshwater species are less than an inch (25 mm) in diameter, are colorless and do not sting. These are the large, often colorful, jellyfish that are common in coastal zones worldwide. Over the last several decades, there has been a noteworthy interest in the role that these predatory organisms play in marine ecosystems, especially their effects on lower trophic levels [2-8]. Particular responsiveness has been paid to cases where fish species of marketable importance were among the dominant prey consumed by gelatinous predators [9-13].

In some systems, cnidarians and other gelatinous zooplankters can account for a substantial proportion of the daily mortality of the early life stages of fishes. In addition to negatively affecting their prey populations, jellyfishes are also known to alter the seasonal cycle of plankton productivity [14, 15]. Owing to the potential impact jellyfishes have on marine ecosystems, there have been numerous attempts to quantify their consumption of zooplankton or ichthyoplankton, mostly in enclosed bays or seas [16-21].

Today, the Japanese are the leading consumers of jellyfish. Since the 1970s, with increasing demand from the Japanese market, jellyfish fishing has become popular in Southeast Asia. More recently, small-scale exploitation of jellyfish has also commenced in other

countries such as Australia, India, Mexico, Turkey and the U.S.A. In Southeast Asia, jellyfish are fished in the Philippines, Vietnam, Malaysia, Thailand, Indonesia, Singapore and Myanmar. Research suggests that the sea may sustain at least 2 billion different bacteria. But our ability to culture and study them under laboratory conditions is relatively difficult. The microbes associated with marine invertebrates are proved to exhibit outstanding potential for anticancer, antiviral and antioxidant drugs. The aim of this work is to study the microbial diversity in the commonly found jelly fish of Parangipettai coast and to investigate the biochemical properties of selected isolates. And this is the first report on the association of various microbes in the jelly fish.

**Description about the study animal:**

| | |
|---|---|
| **Kingdom** | Animalia |
| **Phylum** | *Cnidaria* |
| **Order** | Rhizostomeae |
| **Suborder** | Daktyliophorae |
| **Family** | Catostylidae |
| **Genus** | Crambionella |
| **Species**: | Crambionella stuhlmanni (Chun, 1896) |
| **Habitat** | Pelagic drifters |
| **Size** | 200mm |

The Bell is slightly pointed, Robust and smooth. Which is edged with furrowed skirt between the lappets the oral arms are tripartite with a small proximal portion and the mouth lets are situated on the central portion and a prominent, smooth, triangular rounded terminal portion is present. There are no filaments. The Bell varies from chocolate color through yellowish brown to milky yellow with purple brow blotches especially peripherally. The oral arms are Colorless to pale pink ion color and the mouth at region is typically spotted with brownish purple.

## Bacterial Association

Bacteria thrive in the dissolved organic carbon (DOC) released by live jellyfish, both in the laboratory [22] and the field [23]. The findings show that all examined jellyfish were carrying filamentous bacteria and that in one case; at least some of these were *T. maritimum*. Similarly, the filamentous bacteria on the gill arch were shown to be *T. maritimum*. The close association between the jellyfish and the lesions on the gill arch suggests that the bacterial lesions were not merely the result of opportunistic infection of an exposed and vulnerable tissue.

A reasonable conclusion is that the jellyfish were responsible for carrying bacteria to the gill arch and for infecting, with *T. maritimum,* the tissue into which they were injecting toxins [24]. Dead jellyfish return energy and nutrients to the food web via rapid degradation and remineralization processes in the water column and on the seafloor by components of the microbial loop. Dead jellyfish, and the chemical plumes [25] around them, constitute habitats and food for both microorganisms and larger invertebrates.

The fate of dead jellyfish and the role of jellyfish as sinks or links in pelagic food webs are determined by a combination of (1) physical and chemical constraints preventing or facilitating break-up, degradation and sinking and (2) species-specific suitability as a substrate for microbes and the specific microbial community composition (cf. Riemann et al. 2006). In addition, other studies [26] have revealed that (3) species-specific palatability to scavenging animals is also of importance for the fate of dead gelatinous matter. We could find no prior appropriate literature describing the presence of bacteria associated with jellyfish, other than some studies about their decay. Thus this study could be the first report on the microbes from jelly fish which may posse's enzyme producing potential.

## 2. Materials and Methods

Samples from the jelly fish Crambionella *stuhlmanni* were obtained from the Parangipettai fish landing centers situated in the east coast of coast India. Ten-gram samples were excised aseptically from those fishes and homogenized in 90 mL of sterile saline water with a Mortar and Pestle and serially diluted up to $10^{-4}$. Then 0.5 mL of each dilution was used to spread in the growth media. Enumeration and isolation of the bacteria were done in nutrient agar. The plates were incubated at room temperature for 3-7 days in sealed polythene bags. The bacterial isolates were further sub cultured on the respective mediums in order to obtain pure culture. Pure isolates were maintained at 4ºC in refrigerator for further studies.

### 2.1 Identification of amylase producing bacteria

#### 2.1.1 Cultural characterization

The isolates were observed under the microscope, the colony morphology was noted with respect to color, shape, size, nature of colony and pigmentation

#### 2.2 Biochemical Characterization

The bacterial isolates were characterized biochemically by Indole test, methyl red test, Voges proskauer test, Simmons citrate test, Catalase test, Oxidase test, Urease test, TSI test, Starch hydrolysis test, H2S production.

**2.3 Screening of Potent Amylase producing Bacteria**

Bacterial isolates were screened for amylolytic properties by starch hydrolysis test on starch agar plate. The microbial isolates were streaked as a line on the starch agar plate and plates were incubated at 37°C for 24 hr. After incubation 1 % of freshly prepared iodine solution was flooded on the starch agar plate. Presence of blue color around the growth indicates negative result and a clear zone of hydrolysis surround the growth indicates positive result. The isolates produced clear zones of hydrolysis were considered as amylase producers and further investigated[27].

**2.4 Amylase Production**

Freshly prepared inoculum was used to inoculate the production medium. For the preparation of inoculum a loop full of bacterial isolate was transferred in 50 ml of inoculum medium contained (g/L) starch 10, peptone 10, yeast extract 20, KH2PO4 0.05, MnCl2.4H2O 0.015, MgSO4.7H2O 0.25, CaCl2.2H2O 0.05 and FeSO4.7H2O 0.01. The flask was loaded on a rotary shaker incubator at a speed of 2000 rpm at 37°C for 24 hours.

Amylase production was carried out by submerged fermentation. 500 ml of the production medium was inoculated with 10 ml of bacterial inoculum. The flask was loaded on a rotary shaker incubator at a speed of 2000 rpm at 37°C for 24 hours. After incubation, fermented broth was centrifuged at 7000 rpm for 15 min in a cooling centrifuge. Supernatant was collected and used for the estimation of amylase.

**2.5 Partial Purification of Amylase Enzyme**

Partial purification of amylase enzyme was achieved by ammonium sulphate precipitation followed by dialysis. 100 ml of cell free extract was saturated with ammonium sulphate up to 80%. The content was incubated over night and centrifuged at 5000 rpm for 20 min. Supernatant was collected and saturated up to 90% with ammonium sulphate. Then the content was centrifuged at 5000 rpm for 20 min and pellet was collected for further analysis.

**2.5 Enzyme Assay for Amylase Enzyme**

Amylase was determined by spectrophotometric method as described by Fisher and Stein.

According to procedure 1.0 ml of culture broth was taken in test tube in duplicate and 1.0 ml of starch substrate was added in test tube. The test tubes were covered and incubate at 35°C for 15 minutes in water bath. Then 2.0 ml DNS reagent was added in each tube to stop the reaction and kept in boiling water bath for 5 minutes. After cooling at room temperature, the absorbance was read at 540 nm by spectrophotometer. A unit of amylase activity was defined as the amount of amylase required to catalyze the liberation of reducing sugar equivalent to one ì mol of D glucose per minute under the assay conditions.

## 3. Results and Discussion

A total 27 different bacterial strains were isolated and characterized on the basis of colony characteristics, microscopic appearance and biochemical tests. Morphological and biochemical characterization of microbes associated in Crambionella *Stuhlmanni* is listed in table 1. All isolates were primarily screened for amylase production by starch plate method. Among 27 isolates, 2 isolates hydrolyzed the starch on starch agar. The strain showing maximum inhibitor zone were selected and further identified by colony characteristics, microscopic appearance and biochemical tests the isolate was identified as Staphylococcus sp. The enzyme, protein and specific activity of the amylase along with the purification fold are listed in table 2.

## 4. Conclusion

The present study is a preliminary investigation on the microbes present in the jelly fish. Based on the results, it can be concluded that the identification of bacteria present in the jelly fish may have several possibilities for future research in the biotechnology aspects. As well, the analysis of microbial biodiversity in marine organisms will also help in isolating and identifying new and potential microorganisms having high specificity for novel compounds.

**Table 1: Partial purification of amylase from Staphylococcus sp.**

| Purification steps | Crude extract | Ammonium precipitation | Dialysis |
|---|---|---|---|
| Enzyme activity (mg) | 0.520 | 0.389 | 0.305 |
| Protein activity(U/ml/mg) | 69 | 26 | 7 |
| Specific activity(U/ml/mg) | 0.003 | 0.009 | 0.019 |
| Purification fold | 1 | 2.66 | 4.9 |
| Yield percentage | 93 | 59 | 48 |

**Table 2: Morphological and Biochemical characterization of microbes associated in Crambionella *Stuhlmanni***

| Biochemical characteristics | Vibrio | Staphylococcus | Lactobacillus | Salmonella | Klebsiella |
|---|---|---|---|---|---|
| Morphology | Curved, Rod shaped | Cocci, Clusters | Rod shaped | Rod shaped | Rod shaped |
| Motility | + | -- | -- | + | -- |
| Grams reaction | -- | + | + | -- | -- |
| Indole | + | -- | -- | -- | -- |
| Methyl Red | + | + | -- | + | -- |
| Voges Proskauer | -- | -- | -- | -- | + |
| Citrate utilization | + | -- | + | + | + |
| Catalase | + | + | + | + | + |
| Oxidase | + | -- | + | -- | -- |
| Urease | + | -- | -- | -- | + |
| Starch hydrolysis | -- | + | + | -- | -- |
| Hydrogen sulphide | -- | -- | -- | + | + |
| TSI | + | + | + | -- | + |

## 5. Research Gaps

The enzyme producing ability of the strains must be carried out in terms of industrial prospective. Jelly fishes are sticky and causes irritation in nature when touched thus research is must in this aspect with respect to the microbial association. The confirmation studies upto Genus level has not been done. The Optimization of temperature, pH, carbon source, nitrogen source and NaCl concentration on amylase enzyme productivity and enzyme activity was not carried out in the study.

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